

sequence of the protein encoded by the DNA is shown in SEQ ID NO: 2. The analysis and search of the sequences was performed by using computer software packages, GenetyxATSQ and Genetyx (both are products provided by Software Development Co., Ltd.).

5 Example 8: Gene expression in the transformant

The transformants obtained in Example 7 were cultured with shaking in a liquid LB medium containing ampicillin (50 µg/ml) at 26°C overnight, and then 0.1 mM IPTG was added to the culture. The shaking culture was prolonged at 30°C for another 4 hours. After culturing, the bacterial cells were collected by centrifuge, washed with physiological saline to
10 remove constituents of the medium. The cells were suspended in 50 mM Tris-HCl buffer (pH 7.5) containing 1% N-acetyl-D-tryptophan and 0.01% 2-mercaptoethanol to make a reaction mixture with total volume of 1 ml. Reaction was performed with shaking at 30°C for 6 hours. *E. coli* JM109 strain was used as a control.

After the reaction was completed, bacterial cells were removed by centrifugation.
15 The amount of amino acid produced in the resulting reaction supernatant was quantified by high performance liquid chromatography with an ODS column (column, Wakosil II 5C18 (φ 4.6 x 250mm) (Wako Pure Chemical Industries); eluate, CH₃CN/50 mM KH₂PO₄/H₃PO₄ (pH2.5, ratio = 2:8); detection, absorbance at 280 nm; flow rate, 1.0 ml/min; column temperature, 40°C). The retention time was 3.3 minutes for D-tryptophan or 8.5 minutes for
20 N-acetyl-D-tryptophan.

The reaction supernatant of the transformant was found to contain 0.15 g/l D-tryptophan; such accumulation of D-tryptophan was not detected in the control supernatant.

25 **WHAT IS CLAIMED IS:**

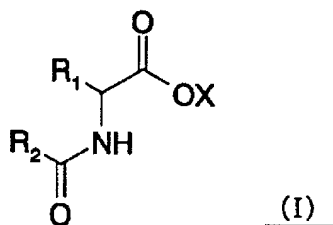
- 1 1. A polynucleotide selected from the group consisting of:
 - 2 (a) a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 1;
 - 3 (b) a polynucleotide encoding a polypeptide comprising the amino acid sequence set
4 forth in SEQ ID NO: 2;
 - 5 (c) a polynucleotide hybridizing to a DNA comprising the nucleotide sequence set
6 forth in SEQ ID NO: 1 under a stringent condition, wherein said polynucleotide encodes a
7 polypeptide having the activity of a D-aminoacylase having the physicochemical properties
8 of (i) and (ii) below; and
 - 9 (d) a polynucleotide encoding a polypeptide having the amino acid sequence set forth
10 in SEQ ID NO: 2 in which one or more amino acid are substituted, deleted, inserted, and/or
11 added, wherein said polynucleotide encodes a polypeptide having the activity of a D-
12 aminoacylase having the physicochemical properties of (i) and (ii) below
 - 13 (i) action: the enzyme acts on N-acetyl-D-amino acids to produce the
14 corresponding D-amino acids and
 - 15 (ii) substrate specificity: the enzyme acts on N-acetyl-D-tryptophan, N-acetyl-D-
16 phenylalanine, N-acetyl-D-valine, N-acetyl-D-leucine, and N-acetyl-D-methionine, but not
17 on N-acetyl-L-tryptophan, N-acetyl-L-phenylalanine, N-acetyl-L-valine, N-acetyl-L-leucine,
18 or N-acetyl-L-methionine.
- 1 2. A polypeptide encoded by the polynucleotide of claim 1.
- 1 3. A vector comprising the polynucleotide of claim 1.
- 1 4. A transformed host cell comprising the polynucleotide of claim 1.
- 1 5. The transformed host cell of claim 4, wherein said cell is derived from *E. coli*.
- 1 6. A method of producing a polypeptide, said method comprising culturing the
2 transformed host cell of claim 4 in a culture, expressing the polypeptide in the cell, and
3 recovering the polypeptide from the culture.
- 1 7. The method of claim 6, wherein said cell is derived from *E. coli*.

1 8. A polynucleotide hybridizing to the polynucleotide set forth in SEQ ID NO: 1 or the
2 complement thereof, wherein said polynucleotide comprises at least 15 nucleotides.

1 9. A method for synthesizing a polynucleotide, said method comprising chemically
2 synthesizing the polynucleotide of claim 8.

1 10. A method for detecting a polynucleotide, said method comprising hybridizing the
2 polynucleotide of claim 8 to a test polynucleotide, and determining whether hybridization has
3 occurred.

1 11. A method for producing D-amino acids, said method comprising contacting a
2 polypeptide with N-acyl-DL-amino acid represented by the formula (I) or its salt:



4 wherein R₁ and R₂ may be identical or different and each represents a hydrogen atom or a
5 substituted or unsubstituted hydrocarbon group; R₂ does not represent a hydrogen atom; and
6 X is H, NH₄, or a metal ion.

1 12. The method of claim 11, wherein R₁ and R₂ in the formula (I) each represents an
2 alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or aralkyl group, or the derivative thereof.

1 13. The method of claim 12, wherein R₁ is a β-methylindolyl, benzyl, thiomethylethyl,
2 isopropyl, or 2-methyl-propyl group; and R₂ is a methyl, chloromethyl, phenyl, or
3 aminomethyl group.